

these parasites were kindly donated by Dr G. Kane, Wellcome Reagents. This material was reconstituted in half the recommended volume of phosphate-buffered saline and was dispensed from a master slide or shallow well by means of the antigen applicator, to large numbers of coated slides. The best results were obtained when Evans blue was used as a counterstain.

ACKNOWLEDGEMENTS

The authors are grateful to Mr G. Ray for constructing the apparatus used in this work, and to Dr C. C. Draper and Mrs D. G. Green for testing the equipment.

Annex

SUPPLIES OF MATERIALS AND REAGENTS

The following sources were used by the authors:

(1) *Conjugates* (fluorescein-labelled antiglobulins): Nordic Pharmaceuticals, Langestraat 57-61, P.O. Box 22, Tilburg, The Netherlands; and Wellcome Reagents Ltd., Beckenham, Kent, England.

(2) *Plastic sprays* (Fluoroglide): Chemplast, 100 Dey Road, Wayne, N.J., USA. (UK agent: Marshall Howlett, 293 Main Road, Sidcup, Kent, England.)

(3) *Ready-prepared coated slides*: These will shortly be available from Shandon Southern Instruments, Ltd, Camberley, Surrey, England.

(4) Details of *Aotus*-adapted strains of *P. falciparum* and *P. malariae*, and of slide-processing apparatus, may be obtained from Dr A. Voller, Nuffield Institute of Comparative Medicine, The Zoological Society of London, Regent's Park, London N.W.1., England.

Aedes aegypti and *Aedes simpsoni* Breeding in Coral Rock Holes on the Coast of Tanzania*

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Aedes aegypti (L) and *Aedes simpsoni* (Theobald) are closely related species of the subgenus *Stegomyia*. Both species are widely distributed throughout Africa (Stone et al., 1959; van Someren, 1968). *Ae. aegypti* and *Ae. simpsoni* are both proved vectors of yellow fever in Africa, particularly in Uganda (Mahaffy et al., 1942; Smithburn & Haddow, 1946) and in Nigeria (Beeuwkes & Hayne, 1931). The recent Ethiopian epidemic of 1960-62 illustrated the potential of epidemics transmitted by *Ae. simpsoni* (Met-selaar et al., 1970). The importance of these two mosquitos in particular as potential vectors of yellow fever makes it imperative to know the full extent of their breeding habits.

During the course of ecological studies on species of *Aedes* in the area of Dar es Salaam, Tanzania, *Ae. aegypti* and *Ae. simpsoni* were both found to be

breeding in holes in coral along the coast. Wiseman et al. (1939) had reported *Ae. aegypti* breeding in such holes on the Kenya coast near Mombasa. However, to the best of our knowledge, the present study is the first to record *Ae. simpsoni* breeding in holes in coral.

Description of the biotope

Msasani peninsula (6°45'S, 39°17'E) is located 8 km north of Dar es Salaam, Tanzania, and extends about 3 km into the Indian Ocean. The entire peninsula is an old elevated coral reef, with a surface up to about 12 m above sea level, which is mostly covered with a thin layer of sandy soil. The underlying rock is coral limestone with numerous cavities and embedded debris such as mollusc shells. The rock surface is frequently exposed and in such places many of the rock cavities are open and collect rain water.

A continuation of the elevated reef at Msasani occurs as the uninhabited offshore Bongoyo Island. On the island the vegetation is rank and luxurious with an almost closed canopy at 10-20 m, and both tree holes and rock holes are common. The peninsula was formerly cleared for the growing of sisal but the plantations have been neglected for several

* This study was supported jointly by US Public Health Service research grant No. CC 00261 from the Center for Disease Control, Atlanta, Ga., USA.

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years and secondary vegetation has rapidly grown up; as there is considerable grazing pressure the secondary vegetation consists mostly of shrubs with a few scattered trees. The most common shrub species are: *Sideroxylon inerme*, *Clerodendrum capitatum*, and *Acalypha fruticosa*. The most common herbs covering the surface, among the holes, are *Tephrosia purpurea* and *Cassytha filiformis*.

Fishermen, shepherds with livestock, and workers digging stones for building material enter the area regularly. Vervet monkeys, several mongooses, rabbits, and even occasional warthogs and bushbucks have been observed in the area.

Observations on the breeding of Aedes

Larvae of *Ae. aegypti* were first observed breeding in November 1968 in the coral rock holes along the Mwasani peninsula when three holes were sampled. During January 1969, samples of soil and debris were scraped from 26 holes, and were taken to the laboratory and flooded; larvae hatching from eggs were reared to adults and identified. Seven (27%) of the 26 holes sampled contained *Ae. aegypti*.

Another similar survey for eggs of *Ae. aegypti* in dry holes was conducted in May 1969. Of 206 holes examined, 46 (22%) contained live eggs of *Ae. aegypti*.

Continued weekly observations revealed that breeding of *Ae. aegypti* in the coral rock holes occurred during the whole of the period from April to October 1969. On only two occasions (5 August and 15 September) were all the sampled holes dry and these were both during the driest part of the year.

Concurrently with the weekly observations on breeding, biting catches were made every one or two weeks from 06.00 h to 10.00 h in the same area. The three human volunteers used as bait readily attracted *Ae. aegypti*. Biting rates ranged to values as high as 8.9 per man-hour.

Females of *Ae. simpsoni* were taken biting in the coral rock hole area during a biting catch on 14 December 1968, and subsequently almost every month from April to September 1969. There were no breeding sites typically associated with this species in the immediate area. Larvae of *Ae. simpsoni* were first found in the coral rock holes on 8 February 1969 and several times subsequently between June and September 1969. The larvae of *Ae. simpsoni* were always found in association with those of *Ae. aegypti*. In addition, larvae of *Ae. simpsoni* were also found several times during the main rainy season,

breeding in the shells of large snails (*Achatina* sp.), which are common in the area but retain water only for a short time.

Bongoyo Island was visited in April and May 1970. Numerous rock holes of the type seen on Mwasani peninsula were full of water and five of the seven sampled contained larvae of *Ae. aegypti*. Two of four water-filled tree holes also contained larvae of *Ae. aegypti* but no larvae of *Ae. simpsoni* were recovered.

In 24 man-hours of hand-net catching, 44 females and 6 males of *Ae. aegypti* and 12 females of *Ae. simpsoni* were caught, while a 4-hour (08.15–12.15 hours) biting catch by three men yielded 3.8 females of *Ae. aegypti* and 2.0 of *Ae. simpsoni* per man-hour.

Discussion

Breeding sites of *Ae. aegypti* in East Africa can be generally divided into two groups: (1) breeding places associated with man, i.e., indoor and outdoor breeding in artificial containers (water-pots, discarded tins, tires, bottles, barrels, buckets, glass jars, etc.); and (2) natural breeding places (tree holes, coconut shells, snail shells, sometimes plant axils, and rock holes). Wiseman et al. (1939) recorded *Ae. aegypti* breeding in holes in coral on the Kenya coast near Mombasa. Since the East African coast is relatively uniform, it is probable that these holes were identical to those observed during the present study. An extensive survey of the coast would probably indicate that *Ae. aegypti* is breeding wherever there are elevated coral outcrops. In view of the abundance of both *Ae. aegypti* and *Ae. simpsoni* on the uninhabited Bongoyo Island, those on Mwasani peninsula should probably be regarded as relict forest populations rather than indicating any invasion of an uncharacteristic habitat.

Ae. simpsoni is generally regarded as a species that breeds in the leaf axils of plants. Gibbins (1942) and Haddow (1948) reported that in Uganda *Ae. simpsoni* oviposited exclusively in leaf axils. Haddow (1948) stated that the most important plants, with regard to *Ae. simpsoni* breeding, in Bwanabwa County, Uganda, were colocasia (*Xanthosoma sagittifolium*), the "Gonja" variety of cultivated banana, and pineapple (*Ananas comosus*). Teesdale (1957) found on the Kenya coast that all local varieties of banana examined were capable of providing breeding sites for *Ae. simpsoni*. Dunn (1926, 1927) and Hopkins (1952) record *Ae. simpsoni* breeding extensively in tree holes. The present work in Tanzania indicates that the breeding sites of *Ae. simpsoni* are pineapple

axils, colocasia, certain bananas, tree holes, coral holes, and snail shells, in order of preference.

Ae. simpsoni is also known to breed frequently in man-made containers in South Africa (Muspratt, 1956). Wiseman et al. (1939) in Kenya reported it breeding in many man-made containers, pools and puddles, rivers and streams, fallen leaves, coconut shells, snail shells, etc. Lewis (1943) even found *Ae. simpsoni* breeding in a rock pool in granite in the Nuba Mountains of Sudan.

The coral rock holes on the Msasani peninsula near Dar es Salaam, Tanzania, are considered to be newly-recorded breeding sites of *Ae. simpsoni*.

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Preliminary Studies on the Development of a Gonococcal Vaccine*

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As it now seems unlikely that gonorrhoea can be contained by chemotherapeutic agents, there appears to be some need for a vaccine for prophylaxis. The development in recent years of better media and better culture techniques has considerably improved the chances of developing such a vaccine. In the present report, we describe in detail the production of a somatic antigen vaccine and the results of a small trial on human volunteers in which the effectiveness of the vaccine was assessed by the measurement of two types of antibodies—one determined by bentonite flocculation and the other by a tissue culture inhibition test.

Materials and methods

Bacterial strains. All the strains used in the study were freshly isolated strains received on chocolate

agar slants from the Ontario Public Health Laboratories, Toronto. Upon receipt, the cultures were streaked on plates with Columbia Blood Agar Base (CBAB) (BBL) and type I colonies (Kellogg et al., 1963, 1968) were picked and again cultured on CBAB plates; they were incubated for 24 hours at 37°C and the resulting growth was harvested in 2% skim milk (Difco) and then lyophilized. All cultures were incubated in an atmosphere containing 10% CO₂. Two strains identified as Canadian Communicable Disease Centre (CCDC) No. 172 and 173 were used for vaccine and CCDC No. 138 was used in the tissue culture antibody inhibition tests.

Culture medium. For vaccine production, *Neisseria* chemical defined medium (NCDM)⁵ described elsewhere (Kenny et al., 1967) was used.

Vaccine production. Tubes of the lyophilized vaccine strains were rehydrated in NCDM and plated on CBAB plates and incubated overnight in an atmosphere containing 10% CO₂. The inoculum was

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⁵ Obtained in powder form from the Grand Island Biological Company, Grand Island, N.Y., USA.